

REMARKS

Applicant's attorney would like to thank the Examiner for the careful consideration given to this case in the Office Action mailed October 23, 2003. In light of the remarks presented *vide infra*, it is respectfully submitted that the claims are in condition for allowance and notice to such effect is requested.

The Examiner objected to the title of the invention for failing to be descriptive. The title has been modified to make it more descriptive by incorporating language from independent claim 1. No new matter has been added.

The disclosure was objected to by the Examiner for containing informalities in the cross references. The specification has been corrected to reflect the current status of the cited application. No new matter has been added.

In general claims 1 has been amended for clarity and antecedent support, dependent claims 2-11 have been amended for antecedent support. In particular claim 1 has been amended to specifically refer to "fluorescent core/shell nanocrystals", as described in applicants' specification pp. 16-17 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0032], which are "functionalized to be water soluble" as described in applicants' specification pp. 3 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0006]. The polynucleotide strands operably bound to the nanocrystal functionalized to be water soluble have been further clarified as those having a predetermined sequence able to hybridize under suitable conditions with complementary polynucleotide strands as described in applicants' specification pp. 3 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0006]. Claims 7 and 8 were amended for clarity to reflect that the fluorescent core could be CdX wherein X is Se, Te, or S and the shell could be YZ wherein Y is

Cd or Zn, and Z is S or Se as described in applicants' specification pp. 24 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0040]. No new matter has been added.

Claim 12 has been amended for clarity to refer to a composition that includes a fluorescent core/shell nanocrystal functionalized to be water soluble and having a plurality of polynucleotide strands of predetermined sequence according to claim 1, that is operably bonded to a target molecule. Claim 13 which depends from claim 12, has been amended for clarity to refer to dendrimers formed between primary and secondary nanocrystals of the present invention. Claim 14, which depends from claim 13, has been amended for clarity to reflect that dendrimer composition of claim 14 provides an increase in the amount of detectable signal obtained from a molecular probe bonded to a target molecule. Support for the amendments are described in applicants' specification pp. 3 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0006]. Claims 15 and 16 were amended for clarity to reflect that the fluorescent core of either the primary or secondary nanocrystals functionalized to be water soluble could be CdX wherein X is Se, Te, or S as described in applicants' specification pp. 24 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0040]. No new matter has been added.

Claims 1-16 stand rejected by the Examiner under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement. The applicants have amended claim 1 to more clearly reflect the hybridizable nature of the polynucleotide strands operably linked to the nanocrystal functionalized to be water soluble of the present invention. The polynucleotide strands of predetermined or known sequence are operably linked to the nanocrystal functionalized to be water soluble and are able to hybridize with complementary polynucleotide strands under suitable conditions as disclosed in applicants'

specification pp. 3 and pp. 8-9 or U.S. Pat. Appl. Pub. 2002/0150905 paragraphs [0006] and [0023].

The present specification discloses how one could link any number or a plurality of hybridizable polynucleotide strands of predetermined or known sequence to a solid support such as functionalized nanocrystals. For example, applicants' specification pp. 39-40 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0058] states: "as illustrated in FIGS. 4A and 4B, the functionalized nanocrystals comprising a plurality of polynucleotide strands are operably linked to the molecular probe via one or more reactive functionalities, wherein the operably linking takes place between a coating of the functionalized nanocrystals (other than the polynucleotide strands) and the molecular probe." and "then reacted in a controlled manner so as to operably link a **finite number** of a plurality of biotinylated polynucleotide strands (e.g., by limiting the ratio of polynucleotide strands: functionalized nanocrystals in the reaction for producing functionalized nanocrystals comprising a plurality of polynucleotide strands).

In applicants' specification pp. 30-31 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0048] applicants recognize that the number of reactive groups and number of hybridizable polynucleotides that may be bonded to the surface can vary depending upon several factors "depending on factors such as the species and/or amount of amino acid (e.g., diaminocarboxylic acid) used to operably link to the capping compound, each operably linked amino acid may have one or more reactive functionalities (e.g., free amino group, free carboxyl group, and a combination there-of) that can be used to operably link to a reactive functionality of a polynucleotide strand (or to a linker)."

Applicants have provided an estimate of the **number of surface groups** on a crystal in the specification pp. 28 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0044], and

one skilled in the art of derivatizing surfaces would know that surface coverage on a nanocrystal may be affected by the amount of time the reactants are exposed to it as well as by the concentration of reactants in contact with the nanocrystals and the reactivity of the reactive functionalities on the nanocrystal. Applicants have provided concentrations of reagents to make the crystals, see applicants' specification pp. 31 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0049] including the use of "mol concentration" of molecules depending upon the size and desired number for operably linking a plurality of molecules to the surface. One skilled in the art would recognize that the number of sites for bonding polynucleotides to a nanocrystal functionalized to be water soluble would be variable and depend upon the size of the nanocrystal and the number and kind of reactive groups. FIG. 1 illustrates a non-limiting example of the structure of the compounds of the present invention. Applicants' specification pp. 36-37 or U.S. Pat. Appl. Pub. 2002/0150905 paragraphs [0054]-[0055] describes thiol and amine reactive functionalities on the surface of the nanocrystals which can be used for binding a plurality of polynucleotide strands to the nanocrystals functionalized to be water soluble.

Applicants' specification pp. 20-21 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0036] describes functionalized fluorescent nanocrystals as those that have enhanced stability and/or solubility in an aqueous environment.

In view of the amendment and arguments, it is respectfully submitted that claims 1-16 are in condition for allowance and that the Examiner's written description rejection should be withdrawn.

Claims 1-16 stand rejected by the Examiner under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. The Examiner has interpreted

Claims 1-16 as encompassing virtually any number of polynucleotides of virtually any length, and that these polynucleotides can be used in virtually any nucleic acid assay for any purpose.

The term "strand" as defined in the applicants' specification pp. 13 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0028] refers to those nucleic acid molecules or polynucleotides sufficient to hybridize with complementary single strands in the formation of a dendrimer. For clarity, the applicants have amended claim 1 to reflect the hybridizable nature of the polynucleotide strands of predetermined sequence operably linked to the nanocrystal functionalized to be water soluble of the present invention. The polynucleotide strands operably linked to the fluorescent nanocrystal functionalized to be water soluble of the present invention are those that are able to hybridize with complementary polynucleotide strands under suitable conditions as disclosed in applicants' specification pp. 3 and pp. 8-9 or U.S. Pat. Appl. Pub. 2002/0150905 paragraphs [0006] and [0023].

Polynucleotide strands of the present invention include those operably bonded to nanocrystals functionalized to be water soluble and those in which "the polynucleotide strand can fulfill its purpose in dendrimer formation." as described in applicants' specification pp. 32 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0050]. Dendrimer formation is the result of hybridization of complementary polynucleotide "strands" as described in applicants' specification pp. 13 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0028]. These polynucleotide strands can also be used to link to a molecular probe or a target molecule as illustrated in FIGS 7-8. Dendrimer formation is utilized to amplify the detectable signal from a functionalized nanocrystal operably bonded to a target molecule or cell as described in applicants' specification pp. 4 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0006] lines 28-32.

The number of reactive groups, linkers, or hybridizable polynucleotide strands on functionalized nanocrystals may be varied as disclosed by applicants pp. 30 or U.S. Pat. Appl.

Pub. 2002/0150905 paragraph [0048] lines 7-14:

“For example, depending on factors such as the species and/or amount of amino acid (e.g., diaminocarboxylic acid) used to operably link to the capping compound, each operably linked amino acid may have one or more reactive functionalities (e.g., free amino group, free carboxyl group, and a combination thereof) that can be used to operably link to a reactive functionality of a polynucleotide strand (or to a linker)”

and pp. 31, U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0049] lines 10-15:

“The nanocrystals were then contacted with a mol concentration of molecules (depending on the size, and desired number) for operably linking a plurality of the molecules to the functionalized nanocrystals”

and pp. 40, U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0058] lines 10-15

“functionalized nanocrystals may be avidinylated, and then reacted in a controlled manner so as to operably link a finite number of a plurality of biotinylated polynucleotide strands (e.g., by limiting the ratio of polynucleotide strands: functionalized nanocrystals in the reaction for producing functionalized nanocrystals comprising a plurality of polynucleotide strands).”

The Examiner cites Zhang et al., Bioinformatics, Vol. 19, No. 1, 2003, page 14.

and Chan (U.S. Pat. Application Publication US 2002/0119455 A1) and expresses the view that the instant specification is silent as to how the skilled artisan is to overcome art-recognized problems of DNA sequencing by hybridization..

In the present invention, nanocrystals functionalized to be water soluble and further functionalized with polynucleotide strands of predetermined sequence may be used to hybridize with target molecules which are polynucleotides like DNA and RNA and therefore may be used in non-isotopic sequence determination. To make and use these nanocrystals functionalized to be water soluble and further functionalized with hybridizable polynucleotide

strands as described by the applicant does not require a teaching by the applicant to solve or describe the computational problems associated with sequencing by DNA hybridization. The hybridization between functionalized nanocrystals with hybridizable polynucleotide strands will occur under suitable conditions to amplify a signal (target molecule detected) without a solution to the problem of hybridization sequence reconstruction.

The Examiner cites Carrico, (U.S. Patent 5,200, 313) to illustrate that the extent and specificity of hybridization is affected by several principal conditions such as base compositions, length, ionic strength, temperature and nucleic acid concentration. One skilled in the art could look to Carrico, (U.S. Patent 5,200, 313) for these conditions, however applicants' specification also describes the effect that these conditions have on hybridization which would enable one skilled in the art to make and use the present invention.

Applicants' specification pp. 32-33 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0051] describes polynucleotide sequences operably linked to nanocrystals functionalized to be water soluble as having extending therefrom a plurality of **polynucleotide strands of "known sequence."** and that "it is apparent to those skilled in the art that the polynucleotide strand may be comprised of a plurality of a single, repeated nucleobase; or a plurality a combination of nucleobases. For example, such a sequence can be made up of a combination of nucleobases (e.g., of more than one type of nucleobase, wherein the nucleobase types comprise A, T, C, G, and U)." Applicants' specification pp. 32 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0050] describes polynucleotide sequences used in the present invention as "The polynucleotide strand is a nucleic acid molecule which is totally or substantially single-stranded and has no particular size, **length** or content limitations, so long as the polynucleotide strand can fulfill its purpose in dendrimer formation." Claim 1 has been

amended to reflect that the polynucleotide strands are of a predetermined sequence able to hybridize under suitable conditions with complementary polynucleotide strands.

Applicants' specification pp. 8-9 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0023] describes the "suitable" conditions under which hybridization occurs as those "under optimal reaction conditions of **temperature**, **ionic strength**, and **time** of reaction which permit hybridization between the desired complementary nucleic acid molecules and that minimize nondiscriminate hybridization (e.g., disfavor non-homologous base pairing)."

Applicants' specification pp. 9-10 or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0024] describes that an "**effective amount**" of secondary dots means that added to a mixture is a sufficient amount of secondary dots to hybridize with primary dots containing complementary polynucleotide strands," and further "an effective amount comprises an amount that would saturate (e.g., bind substantially all available) any specific and available binding sites of its target (e.g., if a secondary dot, bind to all available primary dots containing complementary polynucleotide strands).

In view of the amendments to the claims and the arguments presented showing support that the claims as amended are enabled, it is respectfully submitted that the claims are in condition for allowance.

Claims 1-16 stand rejected by the Examiner under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point and distinctly claim the subject matter which applicant regards as the invention. In the Examiner's opinion, Claims 1-16 are indefinite with respect to what constitutes the meets and bounds of a "nanocrystal." In specification pp. 16 and or U.S. Pat. Appl. Pub. 2002/0150905 paragraph [0032] of applicant's specification, fluorescent nanocrystals are referred to as "nanocrystals comprising semiconductor

nanocrystals or doped metal oxide nanocrystals." Applicant has amended claims 1-16 to specifically refer to fluorescent core/shell nanocrystals functionalized to be water soluble and preserves the right without prejudice to pursue claims to semiconductor nanocrystal or doped metal oxide nanocrystals functionalized with polynucleotides of predetermined sequence in subsequently filed continuing applications.

According to the Examiner, Claim 5 is confusing as to whether it is a product or a method claim. Such confusion arises as a result of the phrase "is used to." Claim 5 has been amended to remove the term "is used to" so that it is clear that the linker operably links a polynucleotide strand of the plurality of polynucleotide strands to the nanocrystal functionalized to be water soluble.

Claims 1-16 stand rejected by the Examiner under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-8 of U.S. Patent No. 6,261,779 B1. In the Examiner's opinion, although the conflicting claims are not identical, they are not patentably distinct from each other because the kit comprises the functionalized nanocrystal claimed instantly.

Claims 1 of the instant invention, and prior to amendment herein, was identical to claim 1 in U.S. Application Serial No. 09/437,076 filed on Nov. 9, 1999. During prosecution of U.S. Application Serial No. 09/437,076, a restriction requirement was made in Office Action Paper Number 9, date mailed 12/20/00, which determined that claim 1 of U.S. Application Serial No. 09/437,076 was patentably distinct, and hence novel and unobvious, from claim 5 and claims 6-27 of the same application. Claims 6-27 were elected in U.S. Application Serial No. 09/437,076 and were subsequently allowed. Claims 6-13 in U.S. Application Serial No. 09/437,076 issued as claims 1-8 in U.S. Pat. No. 6,261,779. Applicants' maintain that claim 1

and claims 2-16 which depend on claim 1 in the instant application, even after amendment, are still patentably distinct from issued claims 1-8 of U.S. Pat. No. 6,261,779 and are entitled to the priority date of U.S. Pat. No. 6,261,779.

In view of the amendments and remarks presented, claims 1-16 are in condition for allowance and notice to such effect is respectfully requested.

CONCLUSION

In view of the remarks and amendments presented above, it is believed that the pending claims (1-16) are in condition for allowance and notice to such effect is respectfully requested. Although Applicant believes no fees are due, the Commissioner is hereby authorized to charge deposit account No. 50-0436 for any fees that may be due in connection with this response. Should the Examiner have any questions regarding these remarks, the Examiner is invited to initiate a telephone conference with the undersigned.

Respectfully Submitted,

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